spp., which jointly comprised around 80% of the detected microorganisms. Others found in the remaining placentas were of the species: Acinetobacter, Bacillus spp., Faklmania, Lactobacillus, Rothia, Micrococcus, Penicillium, Ralstonia, Streptococcus and non-specific aerobic sporulating bacteria.

In contrast, 8 samples of the decontaminated cryopreserved tissue were tested positive for microorganisms with 4 placentas inhabited by C.acnes, 2 by Bacillus spp. while the remaining consisting each of the species Staphylococcus and Ralstonia.

Conclusion Overall, the decontamination measures applied during the preparation of the amniotic tissue can be regarded as effective. We found a significant reduction of the number of microorganisms detected in the amniotic tissue following antibiotic administration.

However, some of the remaining species identified in the processed samples may be considered as contamination during the preparation and testing procedures.

For instance, C.acnes can be considered a result of secondary contamination due to incorrect handling. Species such as Bacillus most likely managed to endure the decontamination procedures.

Possible transmission of SARS-CoV-2 from donors to recipients via cornea grafts is still a concern of the transplantation community. Current recommendations are to avoid corneal transplants from donors with ongoing SARS-CoV-2 infection or those recently exposed to it. During pandemic period in Croatia 21/1113; (1,9%) corneas were procured from donors positive for SARS-CoV-2 by postmortem naso-pharyngeal swabs. That tissue was discarded. Due to the lack of knowledge about the infectivity of such corneas, we started prospective study of SARS-CoV-2 presence in cornea tissue from donors who were SARS-CoV-2-positive upon tissue procurement. In nasopharyngeal swabs of post mortem positive donors cycle threshold values of viral copies were high (CT>34), indicating that there was small number of viral particles in infected donors that could have impact on negative results in tested tissue.

Conclusion Our data suggested that corneas may not be SARS-CoV-2 permissive if the donor was postmortem positive. Further research is required to gain more coherent insight into SARS-CoV-2 transmission via corneal transplantation.

Purpose We evaluated the suitability of 2% human platelet lysate (2%HPL) to replace 2% fetal bovine serum containing medium (2%FBS) for the xeno-free organ culture of human donor corneas.

Methods 32 human corneas unsuitable for transplantation from 16 human donors (age 69.3±15.7 years) were collected. 38.5±17.1 hours after death. They were first cultured in 2% FBS containing medium for 3 days (time point TP1), then evaluated by phase contrast microscopy (endothelial cell density (ECD) and cell morphology). Following an additional 25-days culture period (time point TP2) in either 2%FBS or 2%HPL medium the pairs were again compared by phase contrast microscopy (ECM and morphology), stroma and Descemet membrane/endothelium (DmE) were processed for next generation sequencing (NGS).

Results ECD did not differ between the 2%HPL and 2%FBS group at TP1 (p=0.87). At TP2 the ECD was higher in the 2%HPL group (2179±288cells/mm2) compared to 2%FBS (2113±313cells/mm2; p=0.03), and endothelial cell loss was lower (ECL hPL=-0.7% vs. FBS=-3.8%; p=0.01). There were no significant differences in cell morphology, neither between TP1 and 2 nor between 2%HPL and 2%FBS. NGS showed the differential expression of 1644 genes in endothelial and 217 genes in stromal cells. 2%HPL led to the upregulation of cytoprotective, anti-inflammatory and anti-fibrotic genes (e.g. HMOX1, SERPINE1, ANGPTL4, LEFTY2, GADD45B, PLIN2, PTX3, GFRA1/2) and the downregulation of pro-inflammatory/apoptotic genes (e.g. CXCL14, SIK1B, PLK5, PPP2R3B, SLURP1, FABP5, MAL, GATA3).

Conclusion 2%HPL is a suitable xeno-free substitution for 2% FBS in human cornea organ culture, inducing less ECL and potentially beneficial alterations in gene expression.
Purpose Human corneas preserved in bioreactor (BR) are characterized by not only a better endothelial viability, but also a more differentiated and stratified epithelium compared to corneas preserved in organoculture. By using corneal preservation in BR, we aimed to analyze the respective contribution of corneal (C), limbal (L), and conjunctival (Conj) epithelia in corneal epithelial regeneration.

Methods Five pairs of corneas from body donation to Science were used with a death-to-collection time <20 hours. A 3- to 5-mm-wide conjunctival flap was kept intact. Five patterns were set up by complete mechanical removal of 1, 2, or 3 epithelia (-): C-L+Conj+, C-L-Conj+, C-L+Conj−, C+L-Conj+, C-L- Conj− (control) (n=2 for each pattern). The L epithelia was destroyed by scraping and thermocoagulation. Corneas were then kept in BR (21mmHg, 2.5μl/min of Corneamax Eurobio, 31°C) for 3 weeks to allow epithelial regeneration. The epithelium was then analyzed using immunofluorescence (IF) on flat mounted cornea by targeting CK12 (corneal epithelium) and CK15 (limbal epithelium). Cell nuclei were counterstained with DAPI. Corneal transparency was quantified using a transparemometer.

Results No epithelium was reconstituted in the C-L-Conj− control group. In the other 4 models including the C-L-Conj+ group, the cornea was transparent and covered by a pluristratified corneal epithelium, characterized by CK12 expression. The human cornea observed maintained transparency in contrast to what generally can be observed in the established European culturing system with submersion of the cornea. Final endothelial layer examinations confirmed the presence of viable endothelial cells, as documented during initial corneal bank quality control.

Conclusion With this proof of principle, we confirmed that we can maintain the integrity of the human donor cornea in our modified EVEIT organ culture system. Further investigation, optimization and confirmation will be pursued to meet medical regulations.

Purpose Corneal donor tissue can be used for a number of different reconstructive surgical operations involving the rehabilitation of injured or degraded anterior, posterior and intermediate corneal lamellae.

Potential corneal donor tissues undergo a rigorous screening process including medical evaluation of the endothelial and stromal layers. Depending on this assessment, the tissue’s scope of use is often narrowed down to few types of emergency procedures mainly due to an insufficient number of viable endothelial cells or divergent cell morphology.

In addition to all these limitations, one must not ignore the sometimes critical post-preparation degeneration caused by the submerged culturing process itself, leading to epithelial debridement and stromal edema. All these factors reduce the already short supply of donor corneas. In this study, we aim to optimize this culturing process to avoid tissue degradation.

Methods We used an organ culturing system based on our long established Ex Vivo Eye Irritation Test System (EVEIT) (Spöler et al., 2015). This bioreactor has been modified in size and shape to accommodate human-sized corneal explants. The established mechanisms for supplying the cornea with nutrients and physiologically relevant pressure conditions were adapted to support sterility. Human donor corneas that failed the initial quality protocol and which are released for research were obtained from our cornea bank and inserted in the EVEIT culture system. Corneal integrity was observed during the cultivation period of 19 days.

Results The human cornea observed maintained transparency in contrast to what generally can be observed in the established European culturing system with submersion of the cornea. Final endothelial layer examinations confirmed the presence of viable endothelial cells, as documented during initial corneal bank quality control.

Conclusion With this proof of principle, we confirmed that we can maintain the integrity of the human donor cornea in our modified EVEIT organ culture system. Further investigation, optimization and confirmation will be pursued to meet medical regulations.

Today, split cornea technique is an established procedure and is mostly used for two recipients by combining deep anterior lamellar keratoplasty (DALK) and Descemet membrane endothelial keratoplasty (DMEK) surgeries. However, for some surgical interventions including block excision with tectonic corneoscleral grafting, split cornea procedure is not planned regularly up to now. In the run-up for this procedure, normally a donor cornea with a bigger scleral ring is gained. Nonetheless, the preparation of the tectonic graft for covering the corneoscleral defect after block excision results in a rest donor cornea transplant which is normally too small for further regular size penetrating keratoplasties (PKs) or combined DALK/DMEK surgeries. However, using a modified donor transplant trephination technique, a corneoscleral transplant for regular size keratoplasties can be gained, also after preparation of a tectonic graft for block excision. Herein, we describe shortly this novel donor preparation technique, the differences compared to the standard procedure, possible applications, and the advantages and disadvantages for the first time.